AKT and Oxidative Stress Team Up to Kill Cancer Cells

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AKT, a protein kinase frequently hyperactivated in cancer, plays an important role in cell survival and contributes to tumor cell resistance to cytotoxic therapies. A new study in this issue of *Cancer Cell* shows that AKT also induces the accumulation of oxygen radicals, which can be exploited to selectively kill cancer cells containing high levels of AKT activity.

A major goal in current oncology research aims to comprehensively characterize the molecular alterations driving cancer initiation and progression, which is expected to help develop more efficient targeted therapies. However, it has become clear in recent years that cell regulation mechanisms are highly dependent on cell type and cellular context. For instance, oncogenic signals that normally fuel cancer progression can, in contrast, contribute to tumor suppression early in transformation by triggering senescence or cell-cycle arrest (Yaswen and Campisi, 2007).

The PI3K/AKT signaling pathway is thought to play a prominent role in the initiation and maintenance of human cancer, as many components of this pathway have been found to be mutated or amplified in a broad range of tumors (Yuan and Cantley, 2008). PI3K is a lipid kinase that is activated downstream of Ras small GTPases and receptor tyrosine kinases (RTKs) (Figure 1). Cooperation between PI3K and the protein kinases PDK1 and mTORC2 in turn results in full activation of the nodal serine/threonine protein kinase AKT, also known as PKB. There are three mammalian AKT family members (Akt1, Akt2, and Akt3), which are broadly expressed and play key roles in the regulation of cell growth, proliferation, and oncogenesis (Skeen et al., 2006). Conversely, the lipid phosphatase PTEN functions as a tumor suppressor by antagonizing PI3K activity and inhibiting the AKT pathway.

In contrast to the well-established, protumorigenic role of AKT, Nogueira et al. (2008) report in this issue of *Cancer Cell* the unexpected implication of AKT in triggering the process of cellular senescence, one of the earliest barriers against cell transformation (Collado et al., 2007). The authors focus on a less-characterized cellular function of the PI3K/AKT pathway, the induction of intracellular reactive oxygen species (ROS), which have been associated with pathological effects when accumulated to high levels (Finkel, 2003).

Nogueira et al. show that ROS production by AKT sensitizes primary mouse and human fibroblasts to replicative senescence, the process by which cells stop dividing after a limited number of passages in culture, as well as to Ras-induced premature senescence, in agreement with the role of ROS as mediators of cellular aging (Yaswen and Campisi, 2007). Accordingly, primary mouse fibroblasts deficient in Akt1 and Akt2 (Akt1/2) contained lower ROS levels and were more resistant to passage- or oncogene-induced senescence. Thus, AKT appears to be a novel regulator of life span in mammalian cells, as has previously been proposed for the ERK1/2 and p38 MAP kinases (Collado et al., 2007). These results also imply that AKT activation might only exert its full transforming potential after evading the senescence response induced by ROS, in line with previous reports (Chen et al., 2005).

So, how does AKT activity raise ROS levels? Previous studies have implicated Ras proteins in the regulation of intracellular ROS production, mainly through the ERK1/2 and Rac signaling pathways (Dolado et al., 2007; Trachootham et al., 2006). Nogueira et al. now show that AKT is also a key mediator of Ras-induced ROS by engaging two different mechanisms (Figure 1). One capitalizes on the evolutionarily conserved role of AKT as a stimulator of glycolysis and oxidative metabolism (Plas and Thompson, 2005). The authors show that AKT stimulates oxidative metabolism in the mitochondria concurrent with enhanced oxygen consumption, which indirectly increases intracellular ROS. Importantly, the reduced mitochondrial activity observed in Akt1/2 double-knockout cells does not seem to be a consequence of impaired proliferation, supporting that AKT directly regulates metabolic enzymes.

An additional mechanism by which AKT can induce ROS accumulation involves the direct phosphorylation and exclusion from the nucleus of the forkhead box O (FoxO) family of transcription factors (Figure 1). Nogueira et al. present several lines of evidence supporting that FoxO inhibition is important for AKT-induced ROS accumulation and senescence. They also confirm previous work showing that FoxO induces the transcriptional expression of the antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase and identify sestrin 3 (Sesn3) as a new FoxO target gene with antioxidant activity. Accordingly, Sesn3, MnSOD, and catalase were all expressed at higher levels in Akt1/2-deficient fibroblasts, contributing to their lower levels of ROS. Of note, Sesn3 downregulation restored ROS levels and the senescence response of Akt1/2-deficient cells close to wild-type levels, suggesting a key role for this enzyme in ROS detoxification. In summary, enhanced mitochondrial activity together with reduced antioxidant defenses via FoxO inhibition seems to account for the ability of AKT to induce ROS.

In contrast to primary cells, immortalized and tumorigenic cell lines are generally refractory to ROS-induced senescence, as this response requires regulatory pathways that are usually inactivated during

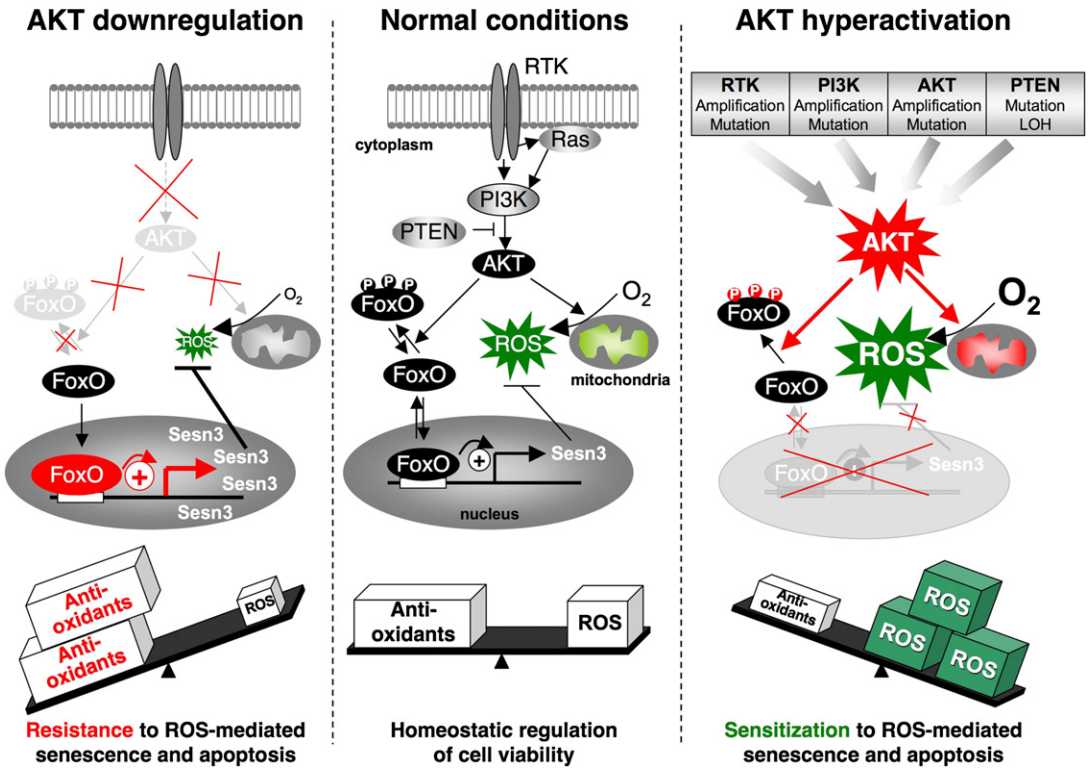

Figure 1. Regulation of Intracellular Reactive Oxygen Species by AKT and Implications for Cell Viability
Under normal conditions (middle panel), AKT increases intracellular reactive oxygen species (ROS) levels by stimulating oxidative metabolism in the mitochondria and also by repressing, via FoxO phosphorylation and sequestration in the cytoplasm, the transcription of genes encoding antioxidant enzymes, of which sestrin 3 (Sesn3) appears to play a prominent role. These mechanisms probably contribute to homeostatic cell regulation. When AKT signaling is downregulated (left panel), ROS levels are reduced due to both decreased mitochondrial activity and increased FoxO-mediated expression of antioxidant enzymes. Conversely, AKT hyperactivation (right panel), as occurs in many cancer cell types, raises metabolic activity in the mitochondria and inhibits FoxO transcriptional activity, resulting in highly increased ROS levels. The upregulation of ROS in turn promotes senescence in primary cells and sensitizes cancer cells to apoptosis induced by oxidative stimuli. RTK, receptor tyrosine kinase; LOH, loss of heterozygosity.

transformation (Collado et al., 2007). Consequently, ROS tend to accumulate in these cells and promote carcinogenesis, except when ROS reach a certain threshold that irreversibly leads to cell death (Trachootham et al., 2006). This double-edged effect of oxidative stress has led to the idea that forced accumulation of ROS might have therapeutic applications (Trachootham et al., 2006); since cancer cells normally contain higher levels of ROS than nontransformed cells, they should be more sensitive to oxidative stimuli.

Importantly, Nogueira et al. also provide evidence, based on both cell cultures and subcutaneous xenografts in mice, supporting the idea that cells with enhanced AKT activity may be selectively killed due to their higher sensitivity to ROS-induced apoptosis. They show that high levels of AKT activity and ROS predispose immortalized murine fibroblasts as well as human cancer cells derived from glioblastoma and ovarian tumors to selective killing by pro-oxidant stimuli, such as hydrogen peroxide or the antioxidant-depleting drug phenylethyl isothiocyanate (PEITC) (Trachootham et al., 2006). The in vivo results of Nogueira et al. are particularly noteworthy, as subcutaneous tumors formed by injection of mice with ovarian cancer cells containing hyperactive AKT were shown to regress completely upon PEITC treatment. Conversely, and in agreement with the well-established prosurvival role of AKT reported by many other groups, AKT hyperactivation induced resistance to drugs that do not function primarily by enhancing ROS levels, such as the topoisomerase inhibitor etoposide.

Given that upregulation of the PI3K/AKT pathway is one of the most prevalent alterations in human cancer, there is much interest in the development of inhibitors to target this signaling pathway (Garcia-Echeverria and Sellers, 2008). However, existing drugs against various components of the PI3K/AKT pathway have shown either limited clinical success or intolerable side toxicity, perhaps due to the conserved role of PI3K/AKT signaling in glucose metabolism in normal cells. Interestingly, the work by Nogueira et al. suggests a new approach to target cancer cells with high AKT activity levels, which exploits the ability of AKT to promote ROS accumulation in order to induce apoptosis via oxidant stimuli. These observations also imply that high ROS levels somehow overcome the prosurvival signaling by AKT, which normally contributes to tumor cell viability. Further studies addressing how ROS evade AKT-induced cell survival might widen the therapeutic opportunities to target apoptosis-refractory human tumors bearing active AKT.

Overall, the study by Nogueira et al. supports previous evidence indicating that therapies based on the induction of oxidative stress might be beneficial for the treatment of human tumors containing

high levels of ROS. This could be further potentiated by combination with drugs that modulate specific signal transduction pathways. Future work should concentrate on identifying appropriate oxidative biomarkers as well as ROS-modulating drugs to address this possibility clinically.

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Suppressing NFAT Increases VEGF Signaling in Hemangiomas

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Infantile hemangiomas represent the most common tumor of endothelial cell (EC) origin, yet the mechanisms regulating hemangioma EC behavior are poorly understood. A new study by Jinnin et al. demonstrates that enhanced VEGFR2 signaling in hemangioma ECs is caused by suppression of NFAT (nuclear factor of activated T cells)-dependent VEGFR1 expression.

Infantile hemangiomas are benign localized lesions, comprised primarily of aberrant endothelial cells (ECs), that appear within the first weeks of life. These lesions go through a proliferation phase during the first 6–10 months and slowly regress over the following 5–10 years. During the involution phase, the vasculature undergoes apoptosis and is replaced by fibrotic fatty tissue. Depending on their location, hemangiomas can cause deformity or life-threatening complications (Barnes et al., 2007). Previously, Olsen and colleagues isolated hemangioma ECs (hemECs) from actively proliferating angiomas of nine unrelated infants and showed that these lines were clonal, arising from a single progenitor cell (Boye et al., 2001). These cells are thought to originate from placental ECs or to be differentiated toward the placental microvascular phenotype (Barnes et al., 2007). Although their origin is uncertain, hemECs have a characteristic expression pattern that is stably maintained in cultured cells and differs from those of normal ECs. They have increased rates of proliferation and enhanced VEGF-mediated migration (Boye et al., 2001). However, until now, the molecular mechanisms driving the aberrant behavior of hemECs remained unknown.

In a recent study, Jinnin et al. (2008) showed that VEGFR2 signaling is constitutively active in cultured hemECs due to decreased VEGFR1 expression. Since both receptors bind VEGF-A, VEGFR1 is thought to negatively regulate VEGFR2 signaling by acting as a decoy receptor for VEGF-A (Olsson et al., 2006). Therefore, constitutive VEGFR2 activity due to suppression of VEGFR1 could explain the increased proliferation and migration of hemECs.

With this in mind, the authors delved further into how VEGFR1 is regulated in hemECs as compared to normal ECs. Because both VEGFR1 (*FLT1*) transcript and protein levels were minimal in hemECs, Jinnin et al. sequenced part of the *FLT1* promoter from all nine hemEC lines. They demonstrated that a region of the *FLT1* promoter contains a binding site for the transcription factor NFAT, providing the first evidence that *FLT1* represents an NFAT target gene. Functionally, this finding was critical to the characterization of hemECs because the authors also demonstrated that NFAT transcriptional activity is lower in hemECs than in normal ECs. Hence, suppression of NFAT-dependent *FLT1* transcription could help drive the enhanced VEGF signaling of hemECs. Looking upstream of NFAT to a cell-surface receptor, Jinnin et al. also found that reduced NFAT activation in hemECs was associated with decreased β1 integrin activity and decreased adhesion to the β1 integrin substrates type I collagen and fibronectin, despite equivalent surface expression of β1 integrin between both cell types.

Previous studies suggested a genetic link between hemangioma growth and